Bull. Mus. natl. Hist. nat., Paris, 4e sér., **17**, 1995,
section B, *Adansonia*, nos 1-2 : 11-20.

Floral heteromorphism in *Dais cotinifolia* L. (*Thymelaeaceae*) : a possible case of heterostyly

M. S. ZAVADA & T. K. LOWREY

Summary : Floral heteromorphism is reported in *Dais cotinifolia* L. (*Thymelaeaceae*) and is associated with an unusual array of ancillary features. The apetalous flowers have three different style morphologies. The upper whorl of stamens in the mid style morph are mid-length, instead of having a whorl of long stamens, as in other tristylous species. There are differences in the morphology of the stigmatic papillae and pollen sculpturing among the three style morphs, however, there is no difference in pollen size. The perianth in the mid style morph is significantly shorter than the perianth in the short and long style morphs. This unusual array of features in what appears to be a tristylous species suggest that floral heteromorphism may be more variable than previously thought. It is also apparent that a more complete survey of tropical *Thymelaeaceae* (and tropical angiosperms in general) for floral heteromorphism is needed.

Résumé : Un hétéromorphisme floral est signalé chez *Dais cotinifolia* L. (*Thymelaeaceae*), associé à une suite inhabituelle de caractères secondaires. Les styles des fleurs apétales présentent trois morphologies différentes. Dans la forme à style moyen, les étamines du verticille supérieur sont de longueurs moyennes plutôt que longues, comme celles d'autres espèces tristyles. La morphologie des papilles stigmatiques ainsi que l'ornementation du pollen varient entre les trois formes de style, mais la taille du pollen reste constante. Le périanthe de la forme à style moyen est notamment plus court que chez les formes à style long ou court. Cette suite inhabituelle de caractères dans une espèce apparemment tristyle élargit la gamme d'hétéromorphisme floral actuellement acceptée. Il est aussi évident qu'une étude plus complète est souhaitable pour les *Thymelaeaceae* tropicales (ainsi que pour l'ensemble des angiospermes tropicaux).

Michael S. Zavada, Dept. of Biology, Providence College, Providence, RI 02918, U.S.A.
Timothy K. Lowrey, Dept. of Biology, University of New Mexico, Albuquerque, NM 87131, U.S.A.

INTRODUCTION

Dais cotinifolia is one of two species in the genus which occurs in subtropical and tropical regions in southern Africa and Madagascar. *Dais cotinifolia* is distributed in the South African provinces of Natal, the eastern Cape and Transvaal, the Transkei, Swaziland, and Zimbabwe (PALGRAVE, 1984 ; VON BREITENBACH, 1974 ; COMPTON, 1976). The second species in the genus, *Dais madagascariensis* Lam., occurs only in Madagascar.

Plants of *Dais cotinifolia* are small trees or shrubs that are three to seven meters in height. The flowers are approximately three centimeters long and vary in color from white to pink to mauve. The inflorescence is an attractive, dense, spherical head subtended by two to six conspicuous shield-like bracts. The perianth consists of a petaloid calyx, which is tubular with five prominent lobes. The ten stamens are in two groups of five differing in length. Preliminary field observations suggested that three style morphologies were present in a population, long, mid, and short. Each of these morphs appeared to be uniform within a plant. This casual observance coupled with the observation that isolated individual plants fail to set viable seed, suggested to us that some form of heterostyly might be present in this species.

Heterostyly is a relatively rare breeding system in plants that occurs in a small percentage of angiosperm families (GANDERS, 1979 ; LLOYD & WEBB, 1992). Heterostyly is recognized as consisting of three associated sets of traits : reciprocal herkogamy, self and intramorph incompatibility, and an array of ancillary floral polymorphisms (BARRETT, 1990). No documented case of heterostyly is known for the *Thymelaeaceae*, however DARWIN (1887) reported a possible case of heterostyly in the family occurring in an unrelated genus in the South Pacific. In this paper we present morphological evidence documenting floral trimorphism involving herkogamy and associated floral polymorphisms. These polymorphisms appear to represent an unusual tristylous condition. Our report sets the stage for the detailed crossing studies needed to establish compatibility relationships indicative or not of a particular heterostyly syndrome.

MATERIALS AND METHODS

Plants were examined in the spring of 1986 and 1987 at the Wilds Nature Reserve within the city limits of Johannesburg, South Africa. Approximately 30 trees are growing in a definable population at the Wilds. The plants at the Wilds consist largely of naturally occurring individuals supplemented with trees imported from other populations in the Transvaal. Twenty-four plants were censused and scored for style morph type at the Wilds. At the Wilds 21 plants were sampled for flowers. Flowers from 5 inflorescences per tree were removed and preserved in FAA (Formalin-Acetic acid-Alcohol). The samples were sorted into three groups on the basis of apparent style and stamen length. Fifty flowers from each morph (7 trees per style morph) were measured for style length, calyx length, gynoecium length, insertion of stamens relative to the calyx mouth, length of short stamens, and length of long stamens. From these data the position of the stigma relative to the calyx mouth, the position of the short stamens relative to the calyx mouth and the position of the long stamens relative to the calyx mouth were calculated. The SAS ANOVA was used to examine the variation among the style morphs using Duncan's multiple range test, Scheffe's multiple comparison test, and the Waller-Duncan k-ratio t test at Alpha = 0.05.

Pollen and excised stigmas were prepared for scanning electron microscopy by immediately fixing live material in the field with 2 % buffered formaldehyde-glutaraldehyde. This was followed in the laboratory by fixation in a 4 % solution of buffered osmium tetroxide. The material was then dehydrated in an alcohol series. Some of the pollen prior to fixation was acetolyzed according to the method of ERDTMAN (1943). Stigmas were critical point dried prior to mounting on SEM stubs. Pollen and stigmas were mounted on SEM stubs with the high vacuum wax Apiezon W-100 according to the method of WACHTEL (1980), coated with gold-palladium and viewed with Hitachi S–450 scanning electron microscope.

Aniline blue in lactophenol was used as an indicator of pollen viability (HAUSER & MORRISON, 1964). Pollen was removed from dehisced anthers from at least five plants with each style morphology and placed in a drop of stain on a microscope slide. One hundred grains were counted from each whorl of stamens from each style morphology. From these counts the proportion of stained grains (presumably viable grains) was determined.

Tests for the presence of self-incompatibility were performed on 8 trees for each style morph. Inflorescences were bagged before anthesis with nylon stockings cut into 30 cm lengths and tied at one end. The emergence of flowers from the bracts was monitored on a daily basis during October and November. At anthesis, pollen from each flower was transferred to the stigmas of the same flowers, different flowers within the same head, and flowers in different heads of the same plant by excising the anthers of both the long and short stamens and dabbing the stigma with the dehisced pollen. Following the self and geitonogamous pollinations each inflorescence was rebagged and tied at the base to exclude pollinators.

Untreated inflorescences were collected to assess natural seed set and serve as the control. Four inflorescences were collected from each of 9 trees and examined for seed set. Pollinations among floral morphs were performed, however due to vandalism we were unable to obtain statistically valid data.

RESULTS

STYLE MORPH RATIOS

The numbers of the short, mid, and long style morphs in the Wilds population do not deviate significantly from 1:1:1.

STYLE

The three style lengths recognized in the field are significantly different and non-overlapping (statistically significant at Alpha = 0.05 for all tests) (Text-fig. 1, Fig. 3). Each individual plant is uniform for a particular style length. The short style averages 22.2 mm, the mid style averages 24.5 mm and the long style averages 29.1 mm in length (Text-fig. 1, Fig. 3). The difference in the length of the style results in differences in the stigma position relative to the calyx mouth (Text-fig. 2, Fig. 1, *1-8*). The short style stigma averages 3.5 mm below the calyx mouth, the mid style stigma averages 1.8 mm above the calyx mouth and the long stigma averages 4.7 mm above the calyx mouth (Text-fig. 2, Fig. 3).

The stigmas of the three style lengths are morphologically distinct. The stigmatic papillae of the short morph are smooth, loosely packed and finger-like (Fig. 1, *3*). The stigmatic papillae of the long style morph are smooth, tightly packed and clavate (Fig. 1, *9*). The stigmatic papillae of the mid form are somewhat intermediate between the short and long form but most similar to the short form, i.e., somewhat loosely packed and finger-like (Fig. 1, *6*), and each papilla is longitudinally ribbed.

STAMENS

Each flower has 10 stamens in two whorls of five, the lower whorl is opposite the calyx lobes and the upper whorl is alternate with the calyx lobes. Both whorls of stamens are inserted on the tubular perianth. The upper whorl is fused to the calyx mouth in all three style morphs. The lower whorl is inserted on the calyx tube about 2.1 mm below the calyx mouth in all three style morphs. There is no significant difference in lower stamen insertion among the three style morphs.

The lower stamens in the short style morph are positioned 1.7 mm above the calyx mouth (Text-fig. 3, Fig. 1, *1-2*). The lower stamens in the mid style morph are positioned 0.2 mm below the calyx mouth and the lower stamens in the long style morph are positioned 0.7 mm below the calyx mouth

(Text-fig. 3, Fig. 3). The whorls of lower stamens in the mid and long style morphs occupy the same position relative to the calyx mouth (Fig. 3).

The upper stamens in the short style form are positioned 4.1 mm above the calyx mouth (Text-fig. 4, Fig. 1, *1-2* ; 3). The upper whorl of stamens in the mid style form are positioned 2.1 mm above the calyx mouth (Text-fig. 4, Fig. 1, *4-5* ; 3). The upper stamens in the long style morph are positioned 2.1 mm above the corolla mouth (Text-fig. 4, Fig. 1, *7-8* ; 3). The positioning of the upper whorl of stamens relative to the calyx mouth in the mid and long morph is not significantly different (i.e., the upper whorl of stamens in the mid style morph occupies a mid position, Fig. 3).

POLLEN

Pollen from all three morphs is spherical, polyporate with crotonoid sculpturing (Fig. 1, *10–12*). Pollen in the short style morph from both the lower and upper whorl of stamens averages 28 µm. Pollen in the mid style morph from the lower whorl of stamens averages 26 µm and that from the upper whorl averages 27 µm. Pollen from the long style morph averages 24 µm from both whorls of stamens. There are no significant differences in pollen size among or within style morphs.

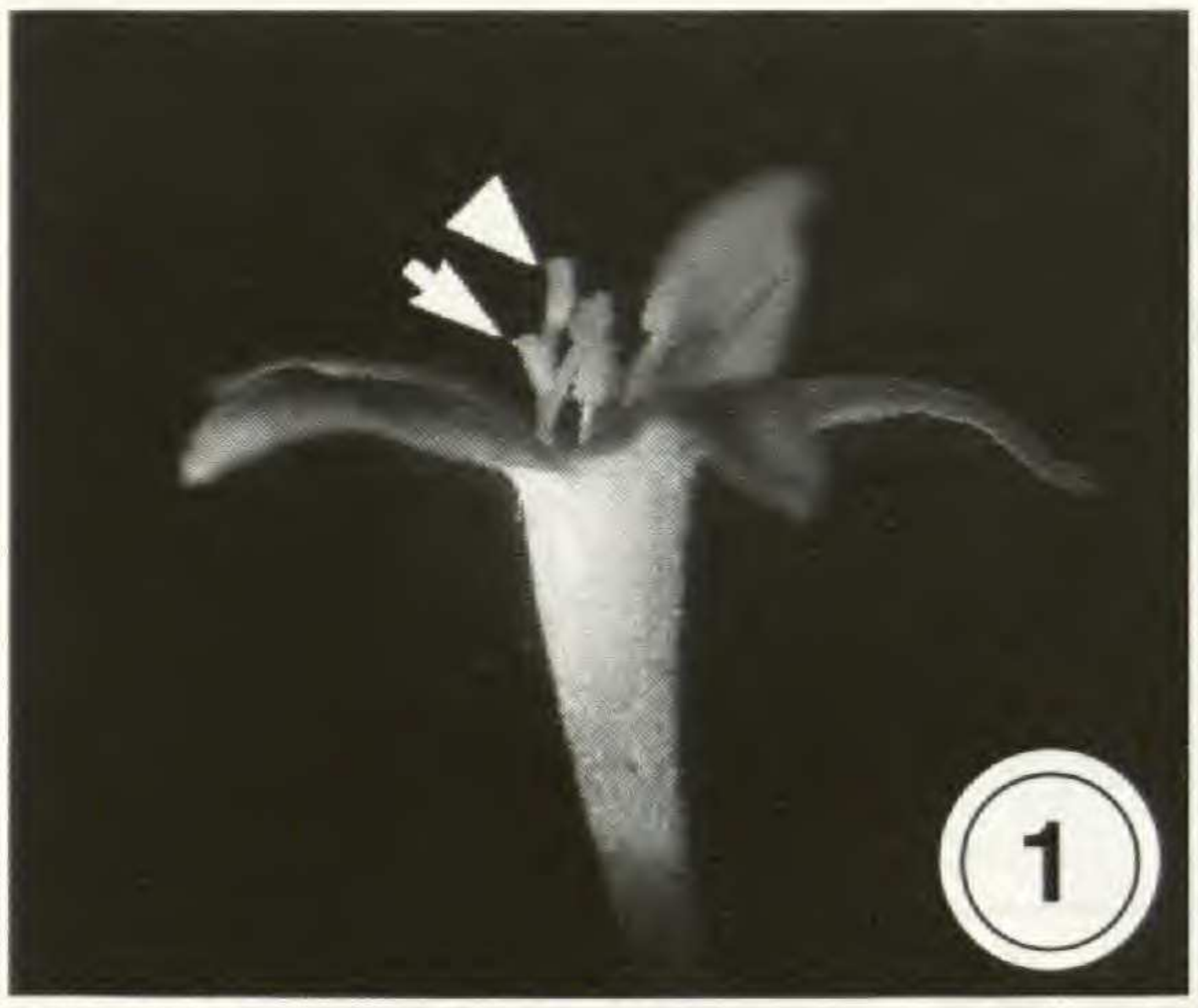
Pollen morphology differs among the three morphs but not between stamen whorls within morphs. The reticulum formed by the crotonoid sculpturing elements in the short and mid style morphs is larger in diameter than in the long morphs. The spaces formed by the reticulum in unacetolyzed pollen are presumably filled with tapetal substances (Fig. 1, *12*). Each crotonoid sculpturing element has a cone-shaped echinate papilla. In the short and mid style morphs the papillae are broad based and blunt (Fig. 1, *10*). In the long style morph the papillae are broad based but more spine-like or echinate (Fig. 1, *11*). Pollen wall structure is similar in all three morphs.

Pollen viability based on staining is greater than 98 % in all style morphs and stamen whorls.

CALYX

The Calyx tube (excluding the calyx lobes) in the short style morph averages 25.7 mm and 24.5 mm in the long style morph. There is no significant difference in the length of the calyx tube between the short and long style morphs. The calyx tube in the mid style form, however, averages 22.7 mm and is significantly shorter than the calyx tube in the short and the long style morphs (Text-fig. 5, Fig. 3).

Fig. 1. — **1**, short style form showing the two whorls of stamens and their relative position with regard to the calyx mouth, long stamens (arrowhead), short stamens (arrow), × 3 ; **2**, short style form with a portion of the calyx cut away to show the position of the stigma (arrow) relative to the calyx mouth, × 3 ; **3**, SEM of the stigma of the short style form showing the long loosely packed, finger-like papillae, × 1,050 ; **4**, mid style form showing the mid whorl of stamens (arrowhead), × 3 ; **5**, mid style form a portion of the calyx cut away showing the position of the stigma relative to the calyx mouth (arrow), × 3 ; **6**, SEM of the stigma of the mid style form showing the finger-like papillae (arrow) which are most similar to the papillae of the short style form (compare Fig. 2, **13** and **16**, and Fig. 2, **14** and **17**), × 1,050 ; **7**, long style form showing the excreted style and stigma and the mid whorl of stamens (arrowhead), × 3 ; **8**, long style form with a portion of the calyx cut away showing the stigma (arrow) relative to the calyx mouth, × 3 ; **9**, SEM of the stigma of the long style form showing the short cylindrical to clavate papillae, × 1,050 ; **10**, SEM of the pollen of the mid style form showing the crotonoid exine sculpturing, and small, short supratectal echinae. The pollen of the mid style form is most similar to the short style form pollen, × 5,500 ; **11**, SEM of the pollen of the long style form showing crotonoid exine sculpturing and the long supratectal echinae, × 6,600 ; **12**, SEM of the pollen from the mid style form. Note that the tectal spaces are filled with tapetal substances, × 6,900.

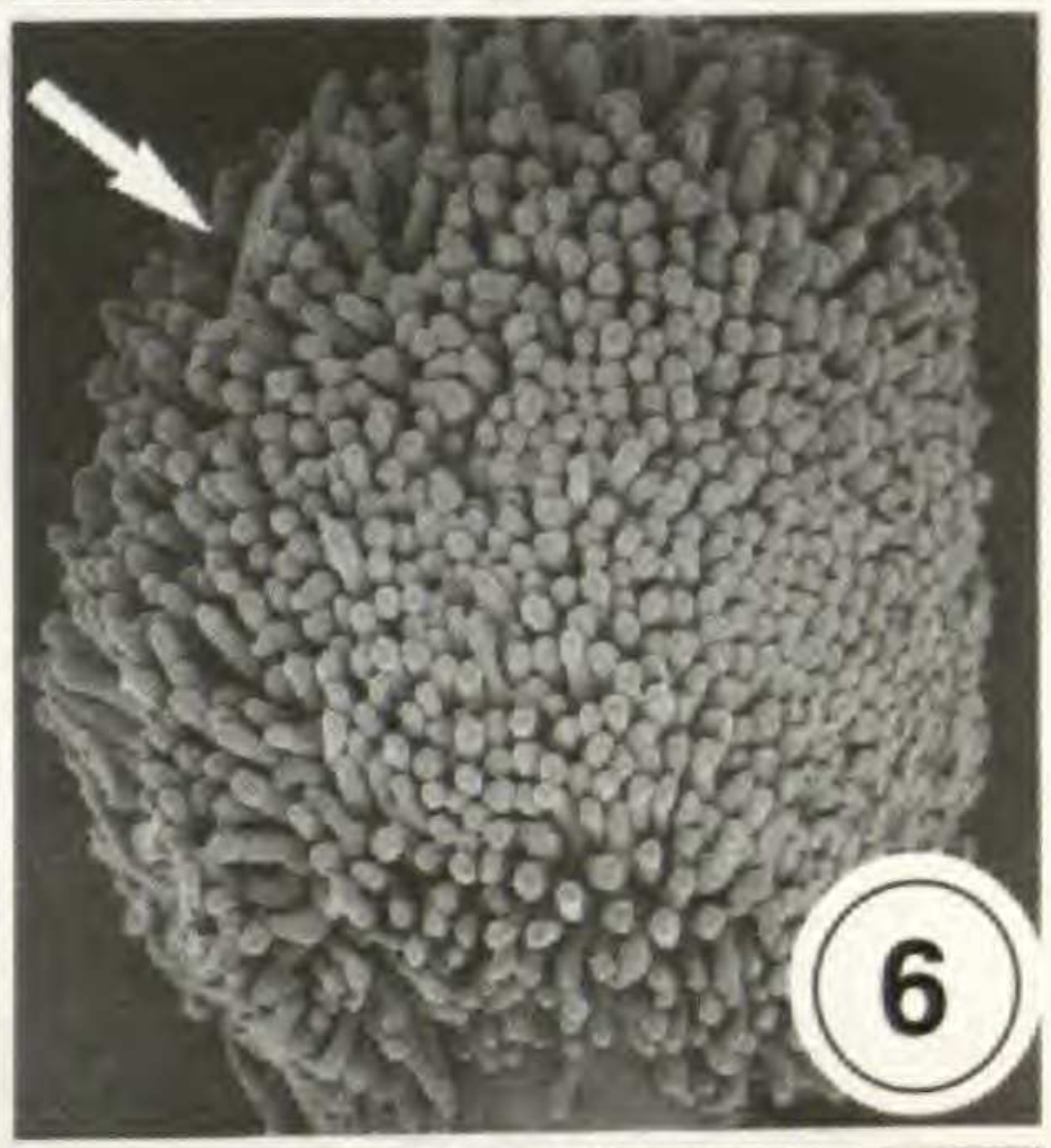

①


②

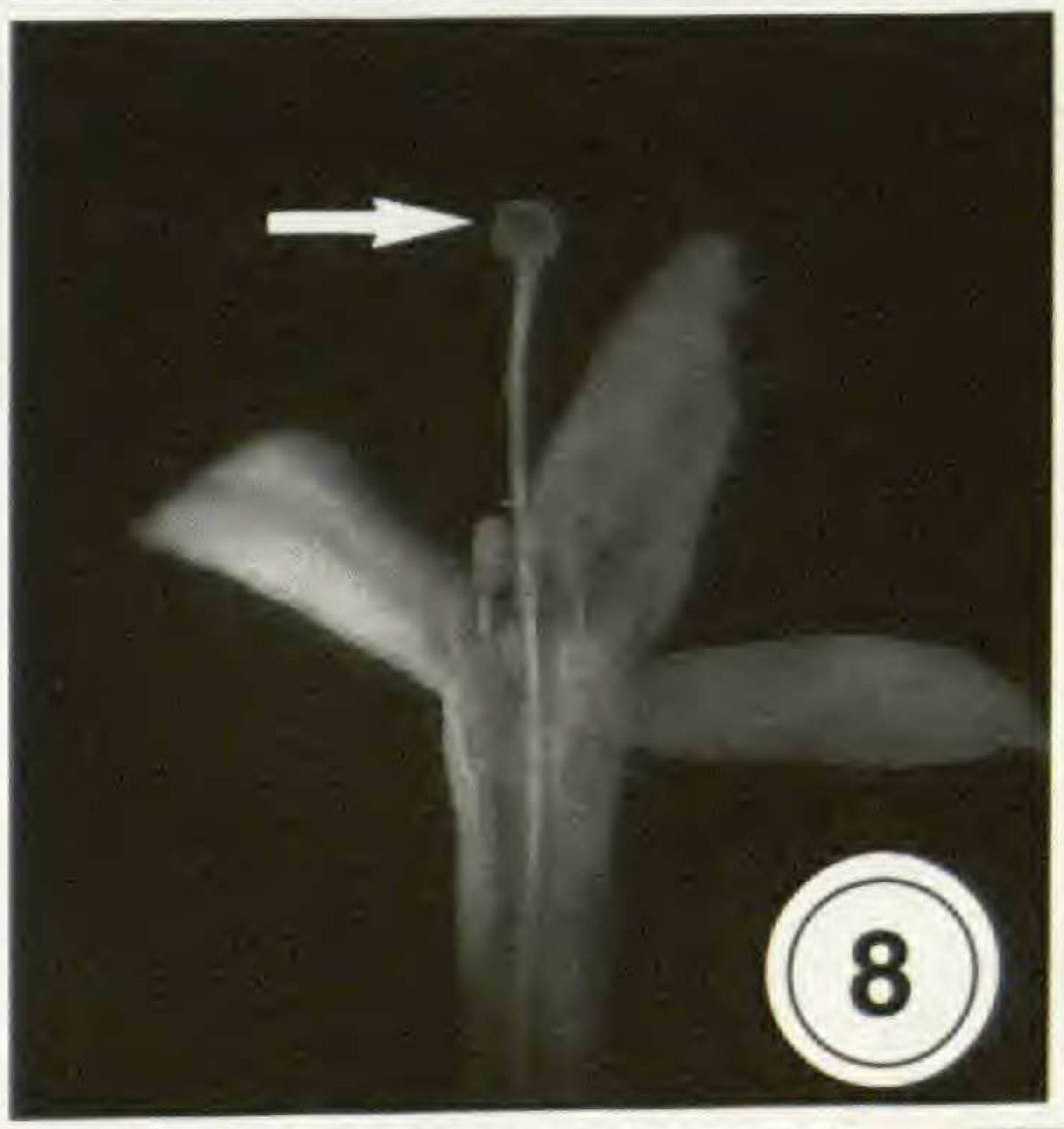

③

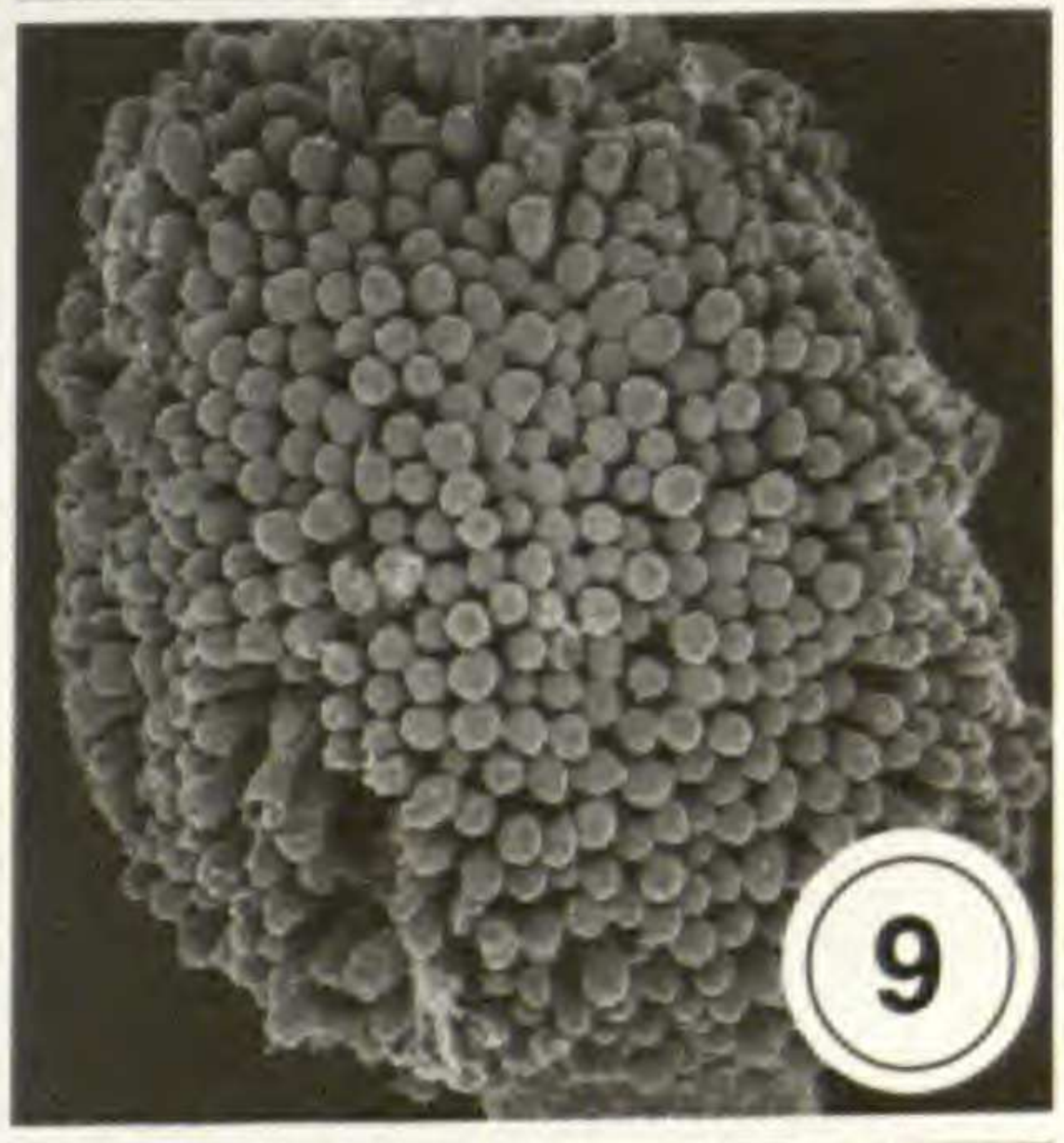

④

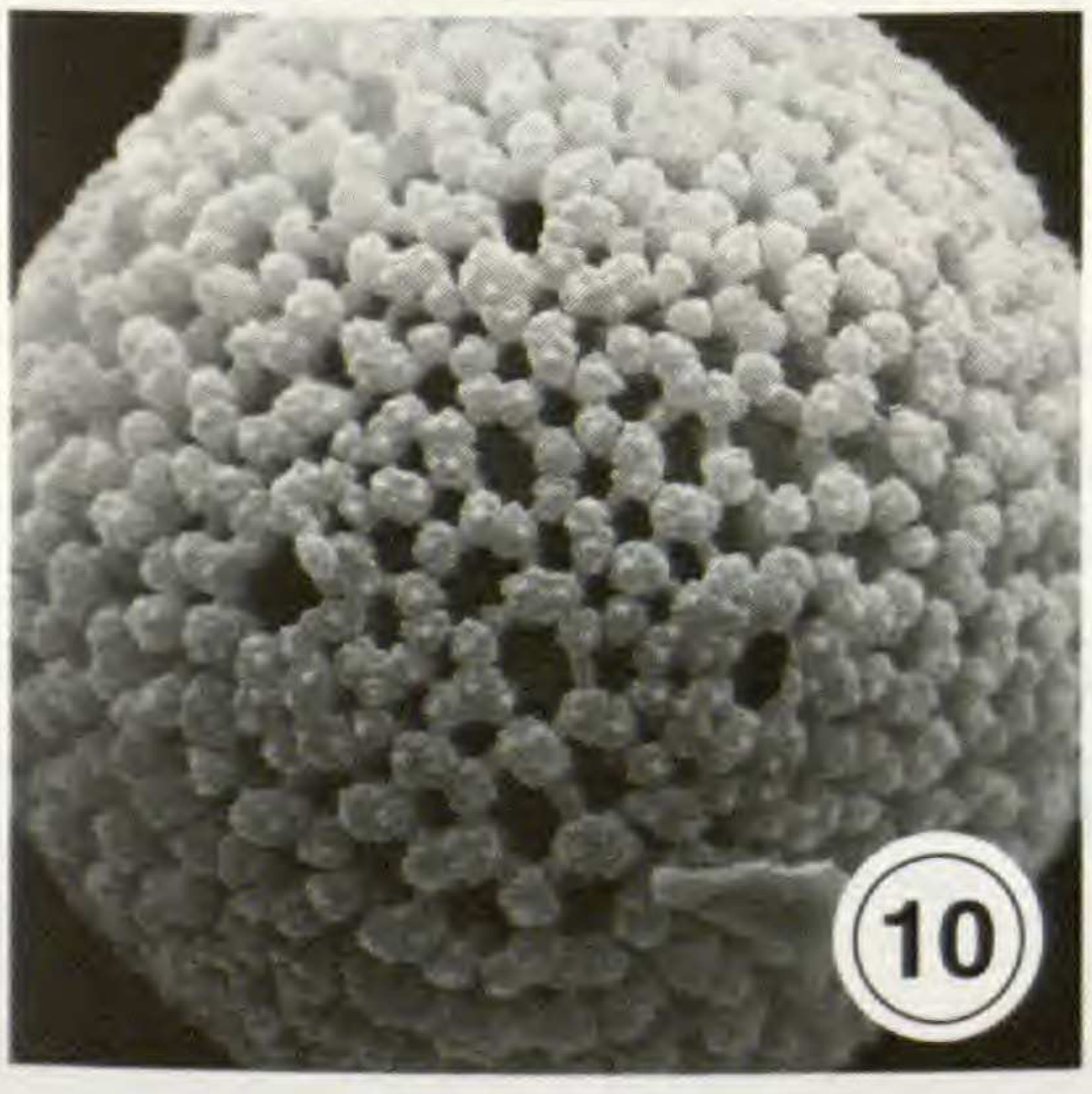

⑤

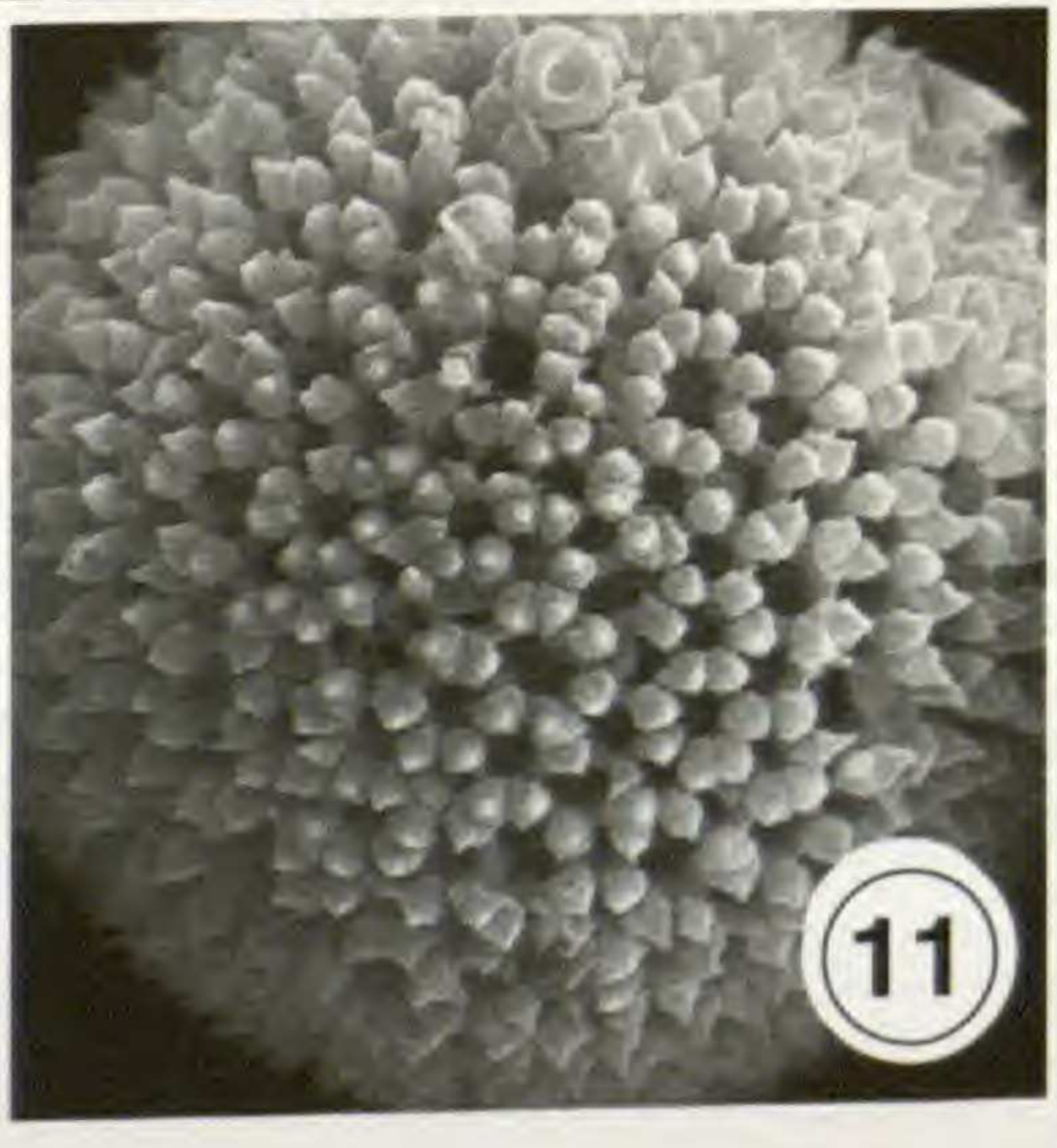

⑥


⑦

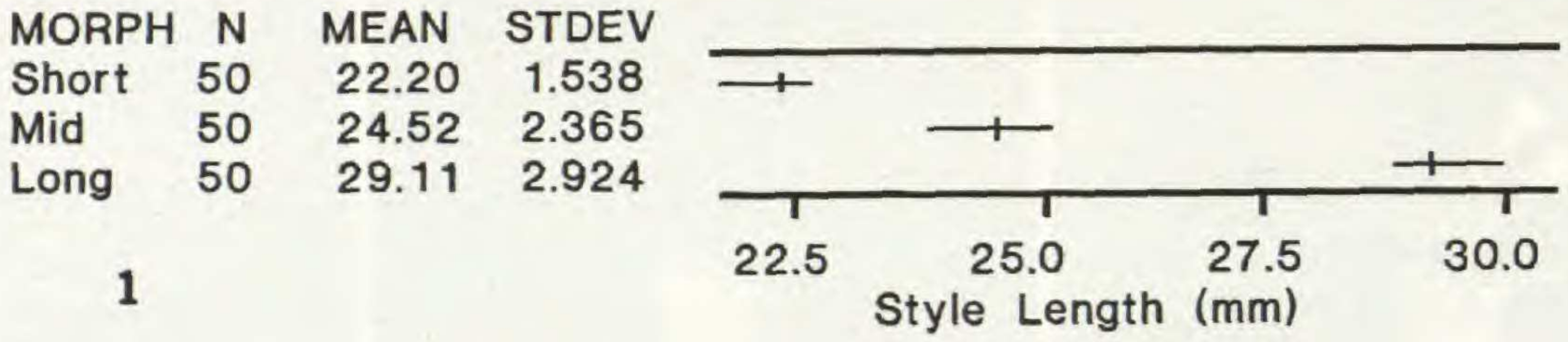

⑧


⑨


⑩

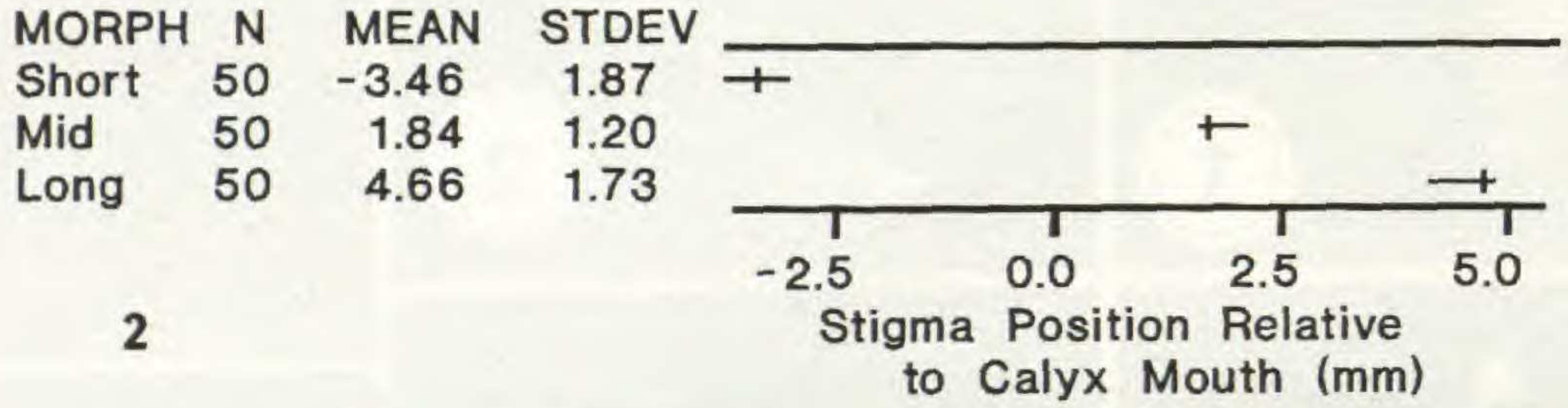

⑪


⑫



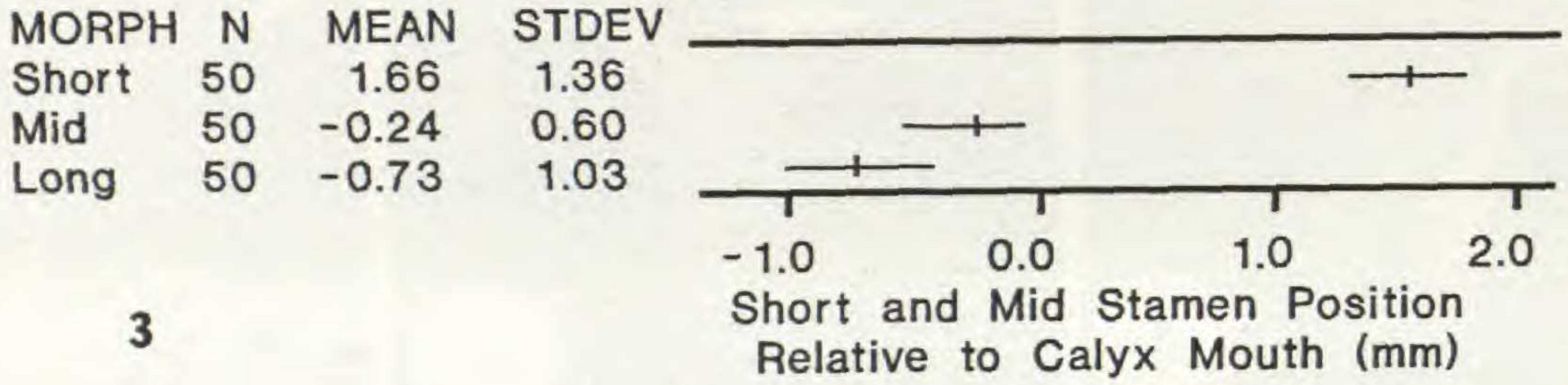
MORPH	N	MEAN	STDEV
Short	50	22.20	1.538
Mid	50	24.52	2.365
Long	50	29.11	2.924

1



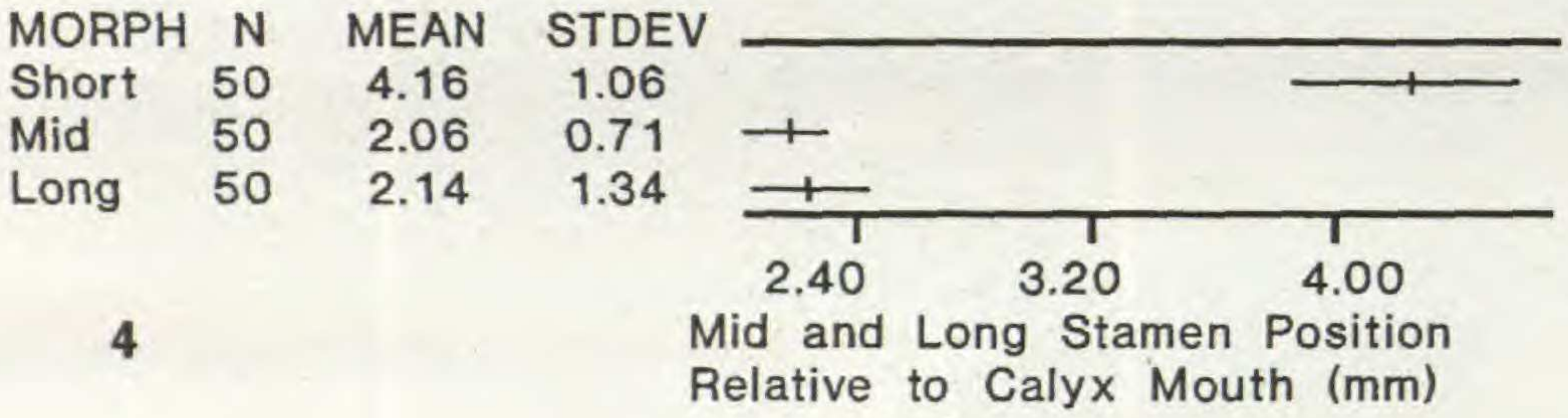
MORPH	N	MEAN	STDEV
Short	50	-3.46	1.87
Mid	50	1.84	1.20
Long	50	4.66	1.73

2



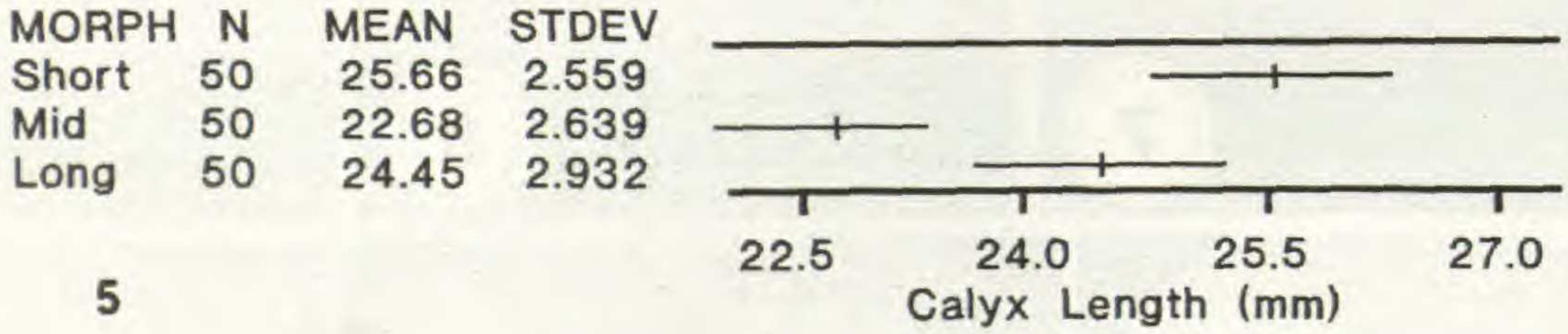
MORPH	N	MEAN	STDEV
Short	50	1.66	1.36
Mid	50	-0.24	0.60
Long	50	-0.73	1.03

3



MORPH	N	MEAN	STDEV
Short	50	4.16	1.06
Mid	50	2.06	0.71
Long	50	2.14	1.34

4



MORPH	N	MEAN	STDEV
Short	50	25.66	2.559
Mid	50	22.68	2.639
Long	50	24.45	2.932

5

Text-fig. 1. — Analysis of variance (ANOVA) of the style lengths showing that the three morphs are distinct and significantly different.

Text-fig. 2. — ANOVA of the stigma position relative to the calyx mouth in the three style morphs.

Text-fig. 3. — ANOVA of the lower stamen position in the mid and long style morphs showing no significant difference in position of this whorl of stamens in the mid and long style morphs.

Text-fig. 4. — ANOVA showing there is no significant difference between the upper whorl of stamens relative to the calyx mouth in the mid and long style morphs.

Text-fig. 5. — ANOVA showing there is a significant difference between the mid calyx length and the short and long calyx length.

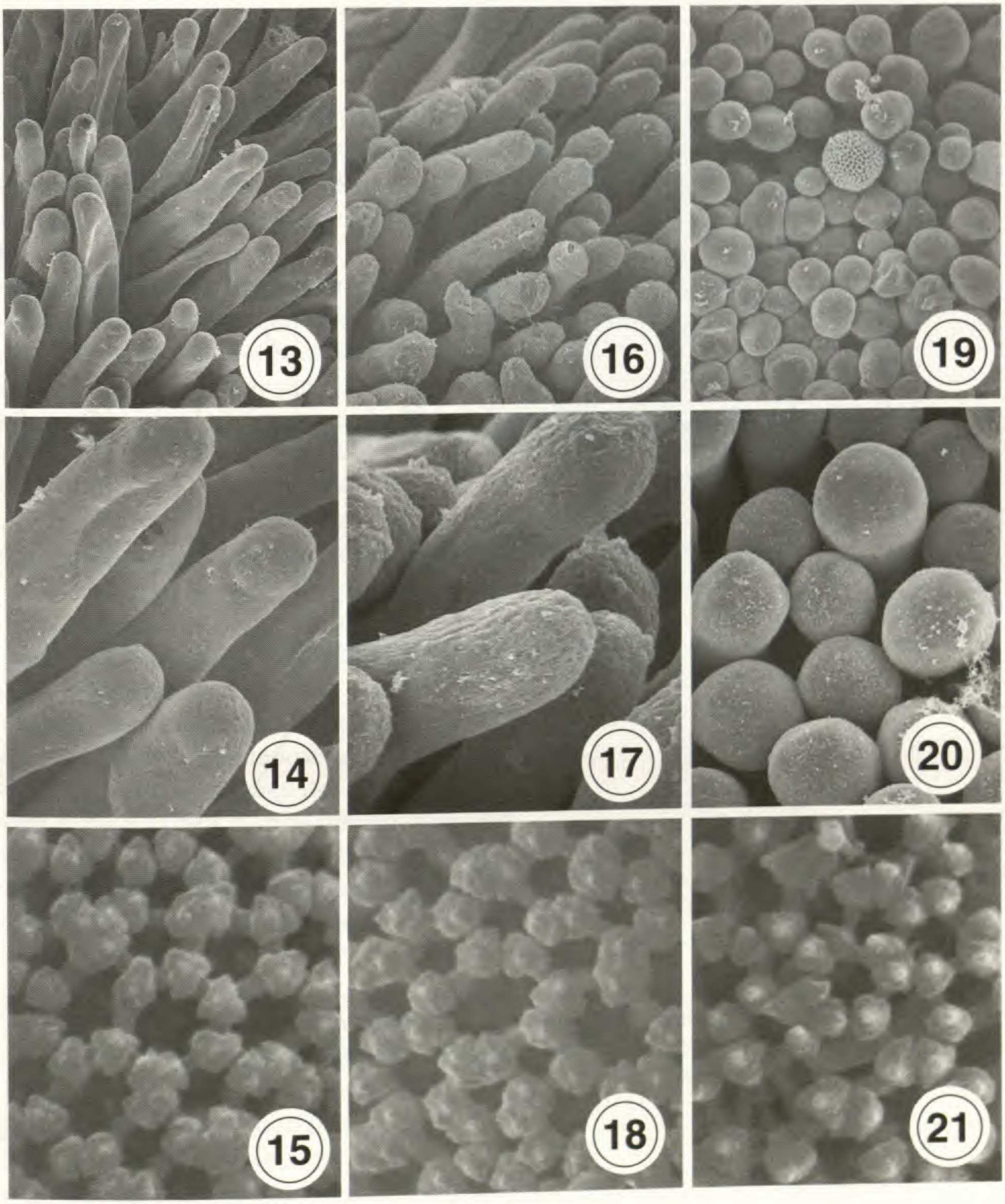


Fig. 2. — **13**, SEM of the long, finger-like stigmatic papillae of the short style form, × 1,500 ; **14**, high magnification SEM of the finger-like stigmatic papillae of the short style form, × 4,500 ; **15**, SEM of the crotonoid exine of the short style form which is most similar to the exine in the mid style form (compare with **18**), × 16,500 ; **16**, SEM of the finger-like stigmatic papillae of the mid style form (compare with the short style form in **13**), × 1,500 ; **17**, high magnification of the finger-like stigmatic papillae of the mid style form, × 4,500 ; **18**, SEM of the crotonoid exine of the mid style form, × 16,500 ; **19**, SEM of the tightly packed, short, clavate stigmatic papillae of the long style form, × 1,050 ; **20**, high magnification SEM of the clavate stigmatic papillae of the long style form, × 2,250 ; **21**, SEM of the exine of the long style form showing the smaller diameter perforation formed by the crotonoid structures and the longer supratectal echinae in comparison to the exines of the short and mid style forms, × 16,500.

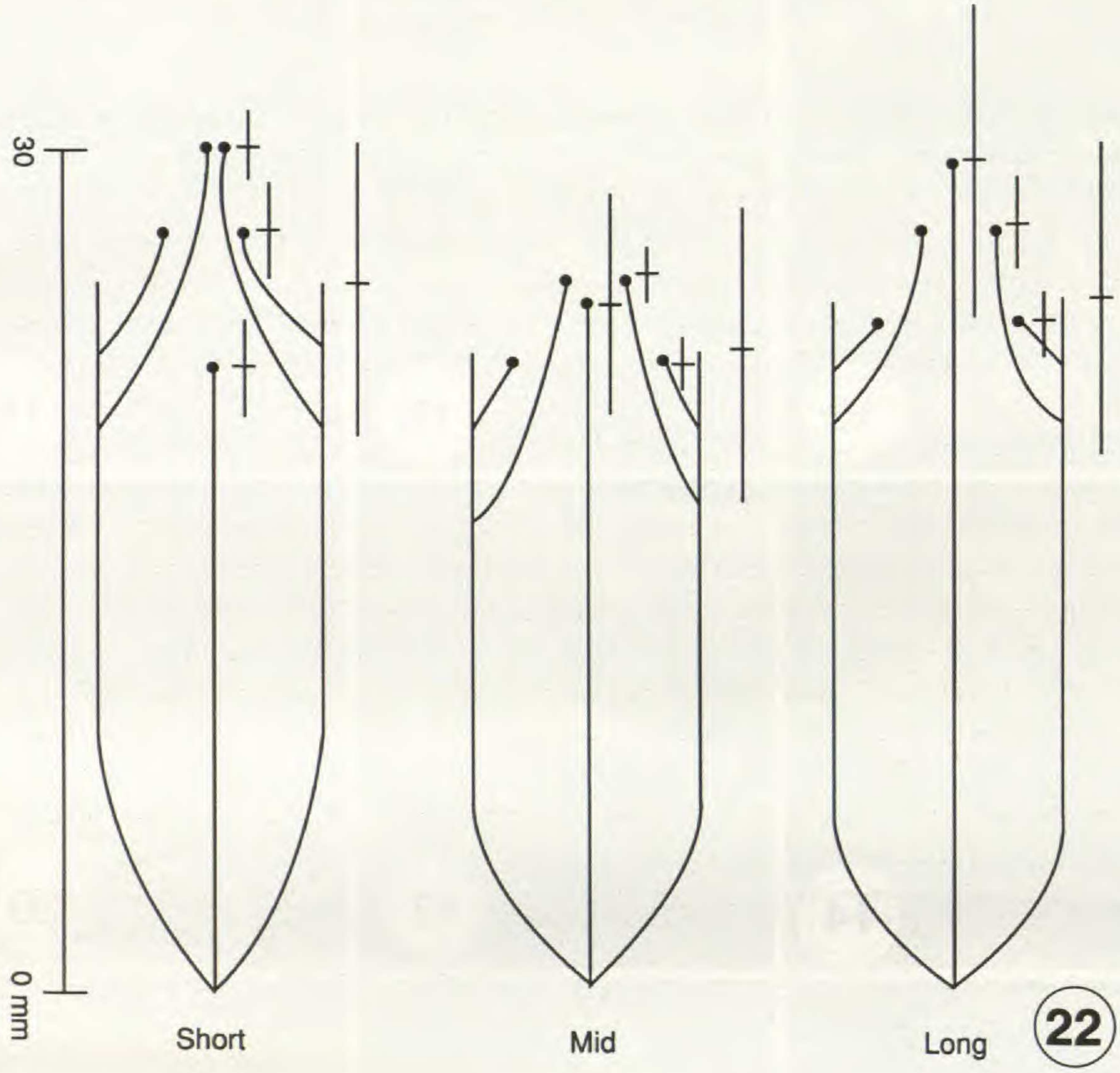


Fig. 3. — Schematic drawing of the flowers of the three style morphs showing relative positions of the styles, stamens and calyx mouth. The mean and range of variation in each floral structure is illustrated to the right of that structure.

SELF-INCOMPATIBILITY

The short and long style morphs are apparently self-incompatible. All self-pollinations resulted in 0 % seed set. Incompatibility in the mid style morph is problematic as one flower head had 5 % of the flowers that set seed while all the other heads used in the treatment set no seed.

DISCUSSION

It is clear that there are three style-length morphs present in the populations of *Dais cotinifolia* that were examined in this study. The occurrence of equal numbers of morph types in both populations provides reassuring evidence that the observed heteromorphism is not due to interpopulation variation nor unusual intra-population variability but rather provides evidence for the existence of tristyly. If we consider *Dais* to be tristylous, there are interesting departures from the norm observed in fully documented tristylous species. Two of the notable exceptions to the " average " tristylous condition are : 1) pollen size and wall sculpturing differences characterize morphs and not stamen levels and 2) the mid morph has the upper whorl of stamens intermediate in length positioned at the same height as the stigma rather than having long stamens, which extend much beyond the stigma. Additionally, the length of the calyx in the mid morph is noticeably shorter than in the other two morphs although differences in perianth morphology among morphs have been noted in other heterostylous taxa (GANDERS, 1979).

There is considerable information in the literature showing variability in the expression of herkogamy and ancillary floral polymorphisms that characterize heterostylous species. The inaccuracy of reciprocal positioning of stamens has been documented for the tristylous *Narcissus triandrus* (LLOYD et al., 1990) and in distylous *Gelsemium* (ORNDUFF, 1970) and *Pentas* (BIR BAHADUR, 1970). Although pollen size trimorphism among stamen whorls is common in tristylous taxa, some taxa show very weak trimorphism (GANDERS, 1979). The lack of pollen differences between whorls in *Dais* may be attributed also to very weak expression of trimorphism. It appears that most heterostylous taxa show some peculiarities in trait expression regardless of whether they are distylous or tristylous.

The individuals within morphs are clearly self-incompatible. The occurrence of some seeds in one head of selfed mids is probably due to contamination. However, the cross compatibility relationships among the different floral morphs remains unknown. All efforts to determine the cross compatibility of the various morphs were frustrated by the vandalization of our controlled crossing experiments in the Johannesburg population. The absence of data about the compatibility relationships and their genetic basis prevents complete confirmation of the occurrence of tristyly in *Dais cotinifolia*. Detailed studies are planned to investigate the nature of compatibility relationships among the morphs in natural populations in Swaziland and Zimbabwe.

Currently, *Dais* is the only member of the *Thymelaeaceae* in which floral heteromorphisms indicative of heterostyly have been documented. The senior author has observed a natural population of the other species (*Dais madagascariensis*), in Ankarafantsika National Park east of Mahajunga, Madagascar. The vegetative and floral morphologies of the two species are generally similar but have several differences. The flowers of *D. madagascariensis* are orange to yellow-orange, 2 cm in length, and monomorphic. Although there are two different staminal whorls in the flowers there is no evidence of heteromorphism involving the stamens or the styles. Despite the sister taxon of *Dais cotinifolia* apparently not exhibiting any heterostylous morphology, there is another report of putative heterostyly in the family. DARWIN (1887) reported a possible case of distyly in *Leucosmia burnettiana* Benth. (= *Phaleria burnettiana* (Benth.) Knuth), a species in the *Thymelaeaceae* from Fiji. This report was based on limited material and has remained unconfirmed. It is apparent that a survey of tropical taxa in the *Thymelaeaceae* for the presence of heterostyly is needed.

LITERATURE CITED

BARRETT, S. C. H., 1990. — The evolution and adaptive significance of heterostyly. *Tree* 5 : 144–148.

BIR BAHADUR, 1970. — Heterostyly and homostyly in *Pentas lanceolata* (Forsk.) Delf. *J. Genet.* 60 : 199–204.

BREITENBACH, F. VON, 1974. — *Southern Cape Forests and Trees*. Government Printer, Pretoria.

COMPTON, R., 1976. — The flora of Swaziland. *Journal of South African Botany*. Suppl. Vol. 11, 684 p.

ORNDUFF, R., 1970. — The systematics and breeding system of *Gelsemium* (*Loganiaceae*). *J. Arnold Arbor*. 51 : 1-17.

DARWIN, C., 1887. — *The different forms of flowers on plants of the same species*. John Murray, London.

ERDTMAN, G., 1943. — *An Introduction to Pollen Analysis*. Chronica Botanica, Waltham, Massachusetts.

GANDERS, F. R., 1979. — The biology of heterostyly. *New Zeal. J. Bot.* 17 : 607–635.

HAUSER, E. J. P. & MORRISON, J. P., 1964. — The cytochemical reduction of nitro blue tetrazolium as an index of pollen viability. *Amer. J. Bot.* 51 : 748–752.

LLOYD, D. G., WEBB, C. J. & DULBERGER, R., 1990. — Heterostyly in species of *Narcissus* (*Amaryllidaceae*) and *Hugonia* (*Linaceae*) and other disputed cases. *Pl. Syst. Evol.* 172 : 215-227.

LLOYD, D. G. & WEBB, C. J., 1992. — The evolution of heterostyly, pp. 151-178 *in* BARRETT, S. C. H. (ed.), *Evolution and function of heterostyly*. Springer, Berlin.

PALGRAVE, K. C., 1984. — *Trees of Southern Africa*. C. Struik Publ., Capetown, 959 p.

WACHTEL, A. W., 1980. — Thermoplastic wax for mounting SEM specimens. *Scanning* 3 : 302.